

Supplementary materials

Title page:

Antarctic marine ciliates under stress: superoxide dismutases from the psychrophilic

***Euplotes focialdi* are cold-active yet heat tolerant enzymes**

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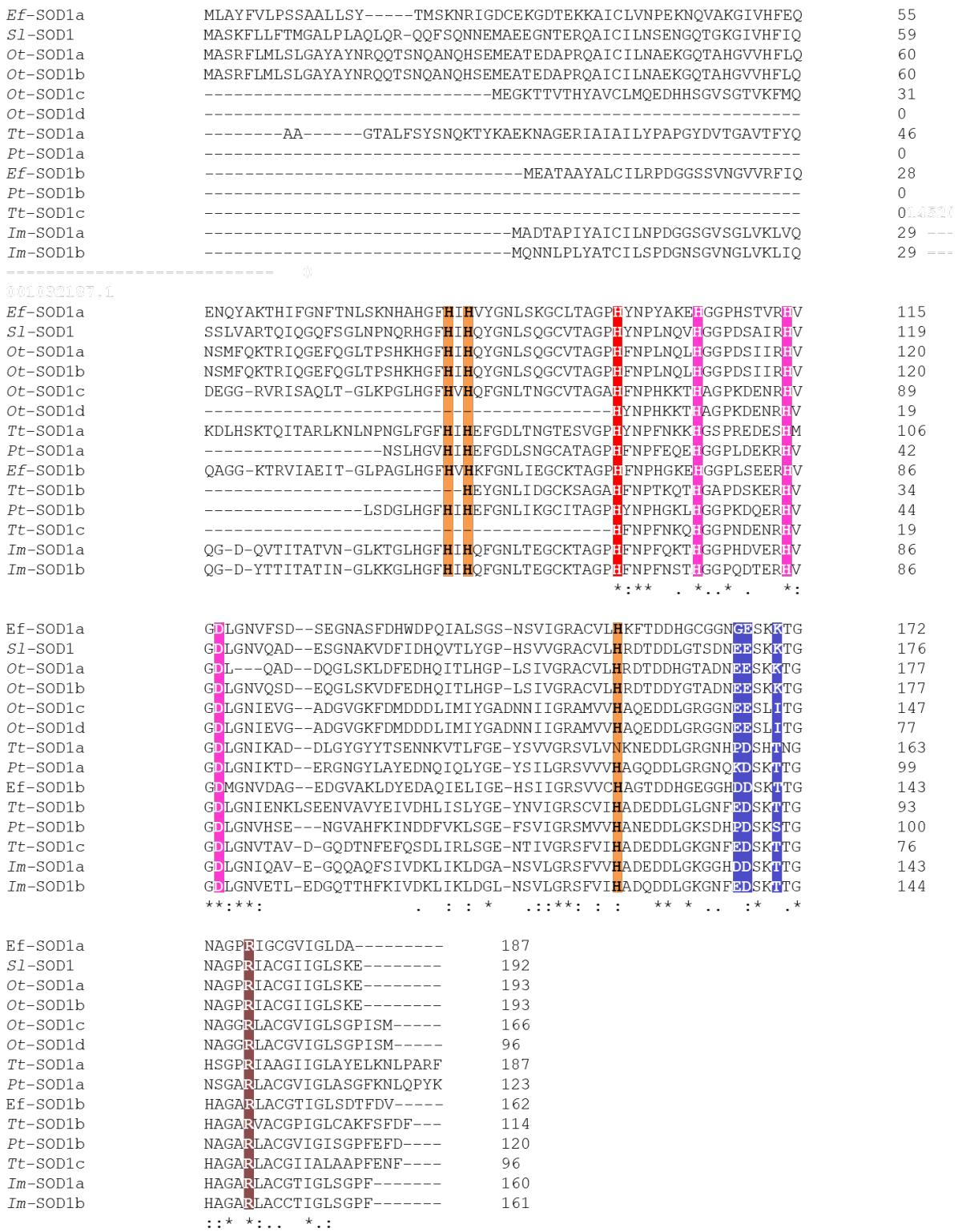


Figure S1. Sequence alignment of SOD1s from ciliate species. Alignment was obtained by ClustalOmega. Residues involved in the coordination of Cu and Zn are highlighted with the same color code used in Figure 5. Residues of the electrostatic triad are in blue, Arg residue following the electrostatic triad is in brown.

Human MnSOD	MLSRAVCGTSR--QIAPALGYLGSRQKHSPLDPYDYGALPHINAQIMQLHHSKHAAAY	58
<i>Ef</i> -SOD2	MLNRVIYKRSQ-ML---FSRAF--SSKVELPALPWEISSLEPTLSAYLLDFHYNKHHQTY	54
<i>Tt</i> -SOD2	-----	0
<i>Ot</i> -SOD2	MLNKAIQNCRQNGLFIQTARCFSSSTKKAELKPLPWDINALEPVLSGNLLDHYNRHHKLY	60
<i>S1</i> -SOD2	-----	0
Human MnSOD	VNNNLNVTEEKYQEALAKGDVTAQTALQPALKFNGGGHINHSIFWTNLSPNG--GGE---P	113
<i>Ef</i> -SOD2	VNNLNLSLQQEGERAIEKGDFETATNLAPLIRFHGGGHINHTFFWHTLASKSQGGGERPSD	114
<i>Tt</i> -SOD2	-----HKIAQLQSGLRFNLLGGHINHAIYWDNLAPVSRGGGVFPDQ	40
<i>Ot</i> -SOD2	VTKFNETLDQLDEAAAKGDHAKIAKLGQNLKFFGGGNYNHTFFWESLAPTKQGGGVQPGS	120
<i>S1</i> -SOD2	-----AQSKNDVAQISKLGQNLKFFGGGNYNHTFFWESLAPTKLGGGNLPGA	47
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Human MnSOD	KGELLEAIKRDFGSFDKFKEKLTAASVGVQGSGWGWLGFNKERGHLQIAACPQNQDPLQG-	172
<i>Ef</i> -SOD2	SGKFGQEVSKTWGSFDNLITDFNTRASPLQGSGWGIVYDKNSKALAYTQTFNQDLITE-	173
<i>Tt</i> -SOD2	NSPLTKAIQEKGWSYENFIQIFNGRTAAIQGSGWGWLGYDTVSKSLKMFELGNQDMPE--	98
<i>Ot</i> -SOD2	DSLLTKHINQTWGSYDKFTKNFSDNTGAIQGSGWGWLVYHKGSKCLEFRPSYNQDLITDY	180
<i>S1</i> -SOD2	DSVLTKHINQTWGSYDKFIANFSAQTAISIQGSGWGWLVYHKGSKTLQYRPSYNQDLITDY	107
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Human MnSOD	TTGLIPLLGI D W W E E AYYLQYKNVRPDYLKAIWNVINWENVTERRYMACKK-----	222
<i>Ef</i> -SOD2	KAGLIPLLNV D M W E E AYYLDYKNARP D FLNNIW D V W N W Q K IEERFNDATKHHHHHH	229
<i>Tt</i> -SOD2	WNSVIPLLT D W W E E AYYLDYQNL R P K YLTE I W K V V N W Q E VERRYLDAIK-----	149
<i>Ot</i> -SOD2	QPDLVPLLN I D W W E AYYLDYKHVKADYLKE I W K V V N W SN D K R L K EAASSQ-----	231
<i>S1</i> -SOD2	QGDLVPLMN I D W W E AYYLDYKHVKADYLKE I W K V I N W DRVE K R L I A Q K Q-----	158
	. : * * : : * : * * * : * : . : * . * . * : * . .	

Figure S2. Sequence alignment of Mn SODs from ciliate species and human. Alignment was obtained by ClustalOmega. Residues involved in the coordination of Mn ions are highlighted in magenta.

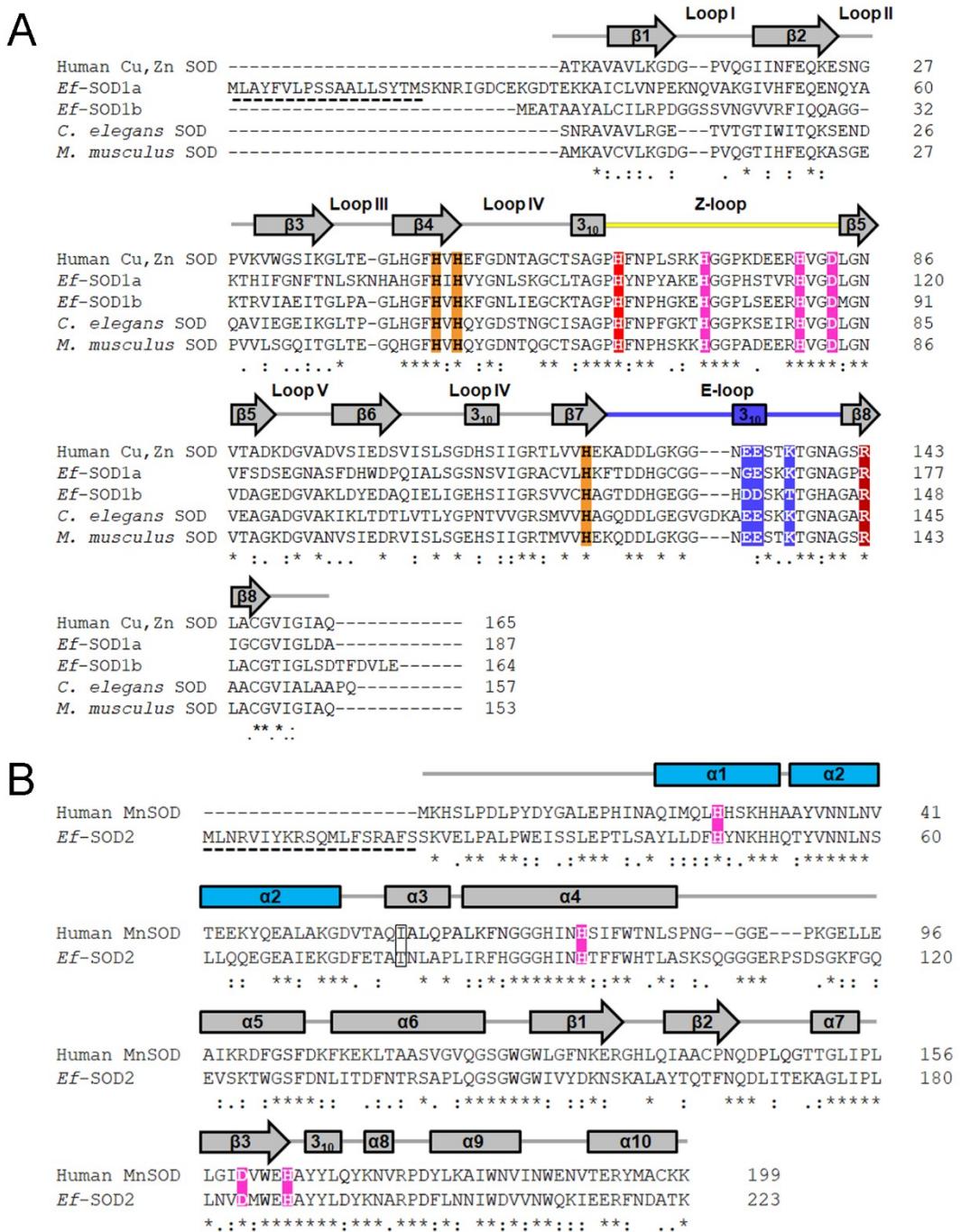
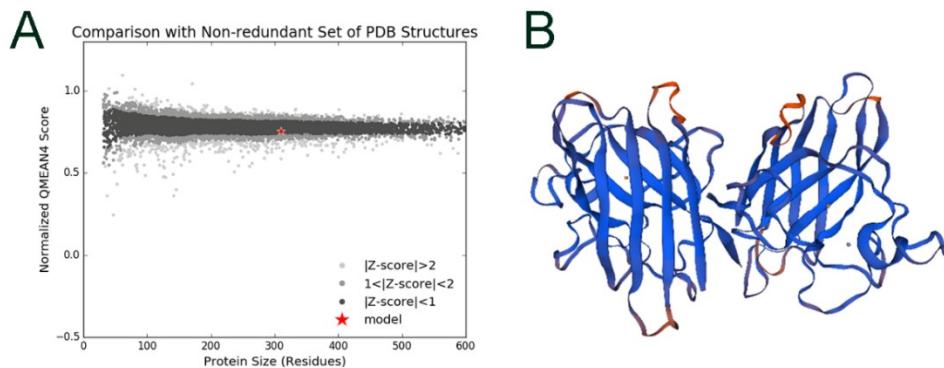
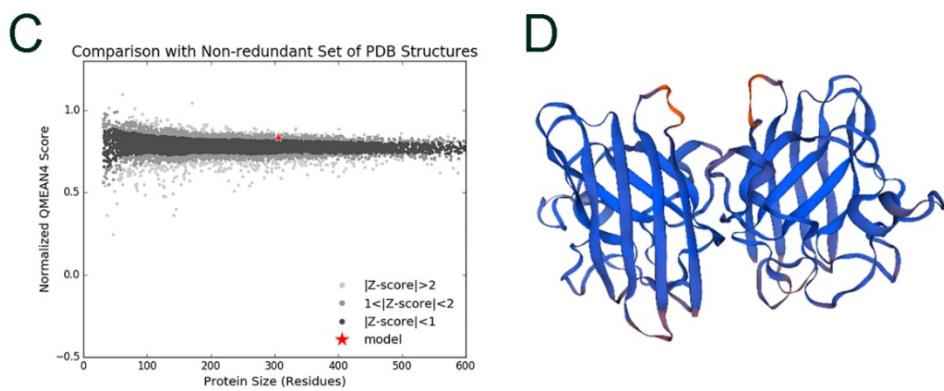


Figure S3. A) Sequence alignment of *Ef*-SOD1s and human Cu,Zn SOD (PDB code: 1NL3), *C. elegans* Cu,Zn SOD1 (PDB: 3KBF) and *Mus musculus* SOD1 (PDB: 3gtt). Color code is the same used in Figure 3A and B. Residues of the electrostatic triad are in blue, Arg residue following the electrostatic triad is in brown. **B)** Sequence alignment of *Ef*-SOD2 and human MnSOD (PDB code: 1VAR). Color code is the same used in Figure 5C. Ile₅₈ in human MnSOD and Thr₇₈ in *Ef*-SOD2 are boxed. Secondary structure elements extracted from PDB files of human SODs are shown. Signal sequences in *Ef*-SOD1a and *Ef*-SOD2 are underlined by a dotted line. The alignment was obtained by ClustalOmega.

Ef-SOD1a



Ef-SOD1b



Ef-SOD2

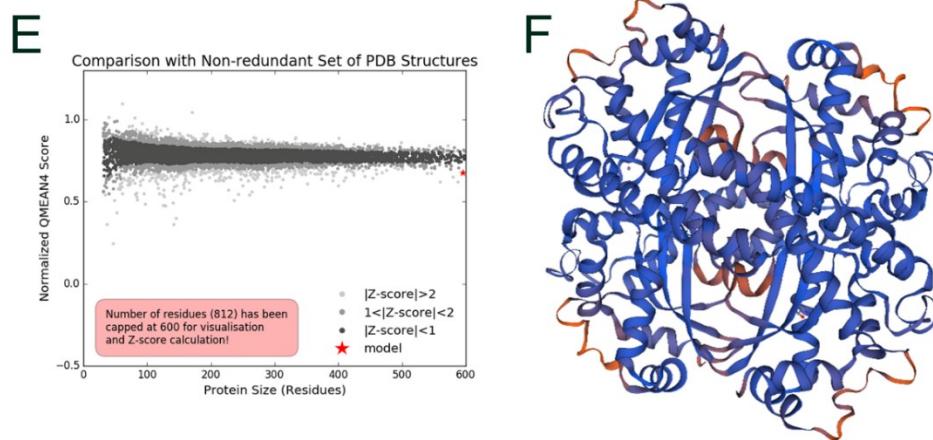


Figure S4. Evaluation of *Ef-SODs* structural models. QMEAN score plot (A, C, E), the normalized QMEAN score is compared with the scores obtained for high resolution crystal structures. Local quality evaluation based on the QMEAN score (B, D, F). Residues with good quality is colored in blue, while the residues poorly modeled are colored in red.

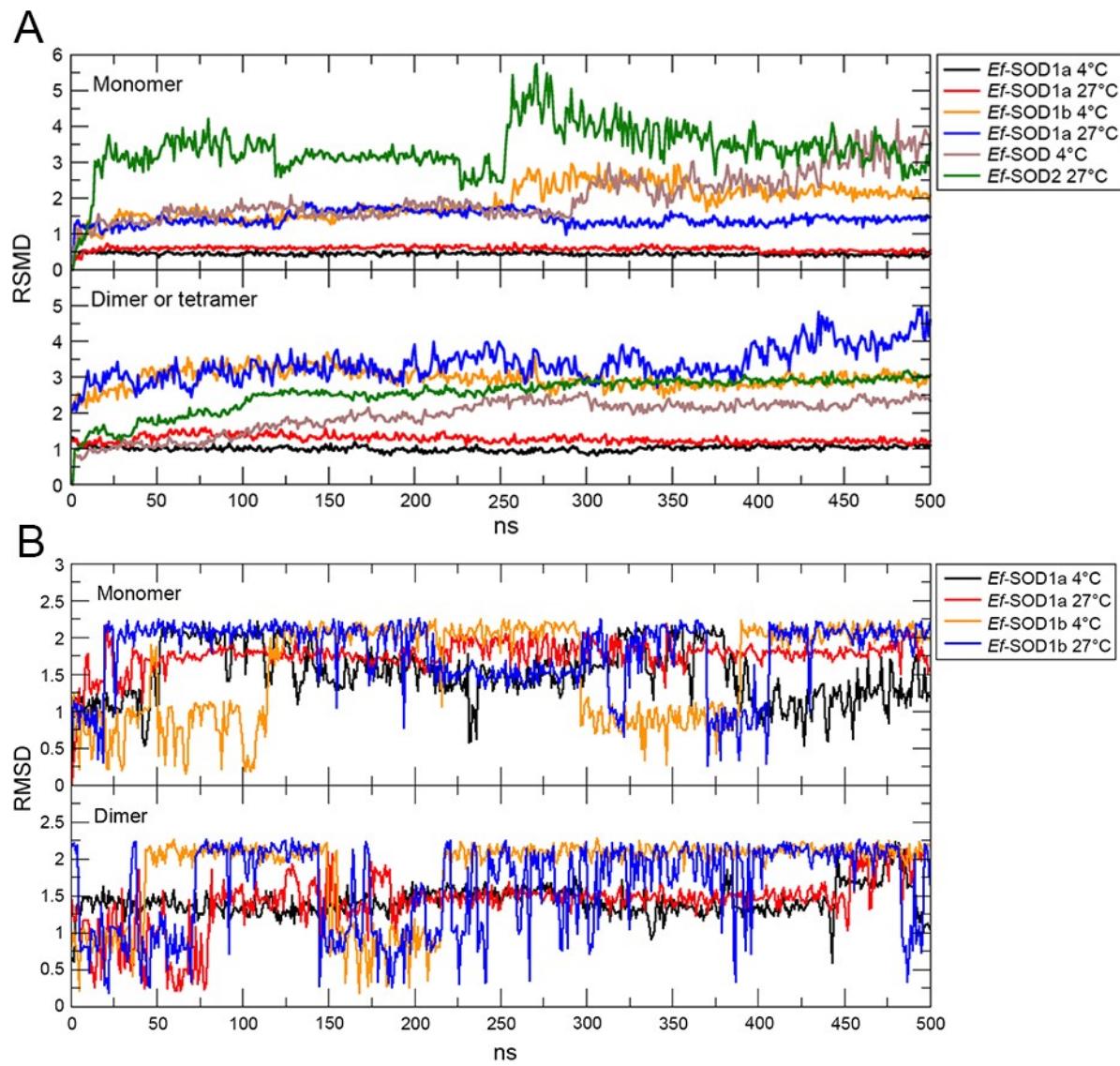


Figure S5. Molecular dynamic simulations of *Ef*-SODs. The local flexibility of *Ef*-SODs was evaluated based on the RMSD profile of ion coordination residues (A) and of Arg in position 177 and 148 in *Ef*-SOD1a and *Ef*-SOD1b (B). Simulations were carried out at 4°C and 27°C. Both monomers and oligomers (dimer for *Ef*-SOD1 and tetramer for *Ef*-SOD2) were considered.

Table S1. Sources and accession number of SOD enzymes reported in phylogenetic tree (Figure 1).

Protein ID	Organism	Accession number
<i>Ef-SOD1a</i>	<i>Euplotes focialii</i>	KF740481
<i>Ef-SOD1b</i>	<i>Euplotes focialii</i>	KF740482
<i>Ef-SOD2</i>	<i>Euplotes focialii</i>	MG575644
<i>EcSOD1a</i>	<i>Euplotes crassus</i>	contig44280
<i>EcSOD1b</i>	<i>Euplotes crassus</i>	contig63897
<i>EcSOD2</i>	<i>Euplotes crassus</i>	contig29865
<i>Sl-SOD1</i>	<i>Styloynchia lemnae</i>	CDW86167.1
<i>Sl-SOD2</i>	<i>Styloynchia lemnae</i>	CDW86249.1
<i>Ot-SOD1a</i>	<i>Oxytricha trifallax</i>	EJY88632.1
<i>Ot-SOD1b</i>	<i>Oxytricha trifallax</i>	EJY70130.1
<i>Ot-SOD1c</i>	<i>Oxytricha trifallax</i>	EJY82908.1
<i>Ot-SOD1d</i>	<i>Oxytricha trifallax</i>	EJY71389.1
<i>Ot-SOD2</i>	<i>Oxytricha trifallax</i>	EJY66799.1
<i>Tt-SOD1a</i>	<i>Tetrahymena thermophila</i>	XP_001007667.2
<i>Tt-SOD1b</i>	<i>Tetrahymena thermophila</i>	XP_001033543.1
<i>Tt-SOD1c</i>	<i>Tetrahymena thermophila</i>	XP_001032187.1
<i>Tt-SOD2</i>	<i>Tetrahymena thermophila</i>	XP_001010506.1
<i>Pt-SOD1a</i>	<i>Paramecium tetraurelia</i>	XP_001445360.1
<i>Pt-SOD1b</i>	<i>Paramecium tetraurelia</i>	XP_001452078.1
<i>Im-SOD1a</i>	<i>Ichthyophthirius multifiliis</i>	XP_004035843.1
<i>Im-SOD1b</i>	<i>Ichthyophthirius multifiliis</i>	XP_004036753.1
<i>Dp-SOD1</i>	<i>Dictyostelium purpureum</i>	XP_00328385401
<i>Fh-SOD1</i>	<i>Flavobacterium hibernum</i>	KIO54302.1
<i>Ns-SOD1</i>	<i>Nesterenkonia sp AN1</i>	EXF25878.1
<i>Pa-SOD1</i>	<i>Planococcus antarcticus</i>	ANU09845.1
<i>Pp-SOD1</i>	<i>Pseudocohnilembus persalinus</i>	KRX07326.1
<i>Ca-SOD2</i>	<i>Cellulophaga algicola DSM 14237</i>	ADV48601.1
<i>Ba-SOD2</i>	<i>Bacillus sp. K2I17</i>	OWT52390.1
<i>Ps-SOD2</i>	<i>Pseudomonas sp. KG01</i>	KMT55131.1
<i>Psa-SOD2</i>	<i>Pseudoalteromonas sp. ANT 506</i>	ALN66863.1
<i>Rh-SOD2</i>	<i>Rhodococcus erythropolis</i>	ORI29058.1

Table S2. Oligomerization state of *Ef*-SODs. Theoretical molecular mass was calculated from the amino acid sequences using ProtParam, and molecular mass was obtained by SEC-MALS analysis.

	Theoretical molecular mass of monomer (kDa)	Molecular mass (kDa)	Oligomerization state
<i>Ef</i> -SOD1a ^Δ	19.4	N.A	Dimer*
<i>Ef</i> -SOD1b	17.6	32.8	Dimer
<i>Ef</i> -SOD2 ^Δ	24.5	95.1	Tetramer

Column: Superose12 10/300 GL (GE Healthcare), mobile phase: NaPi 50mM, NaCl 150mM, pH 7.5.

*Obtained by SEC

Table S3: Primers used for quantitative real time PCR and for the deletion of the signal sequences in the deleted variants (Δ). The *NsiI* restriction site is underlined.

Primer name	Forward Primer (5' – 3')	Reverse primer (5' – 3')
SSU rDNA	GTTACGTCCCTGCCCTTGT	ACCTTGTACGACTTCTCCTTCC
<i>Ef-SOD1a</i>	TTACAGCTGGACCTCACTATAAC	CACAGCCATGGTCATCTGTAA
<i>Ef-SOD1b</i>	CGGAGGAAAGACCAGAGTTAT	GCCAGCAGTCTTACATCCTT
<i>Ef-SOD2</i>	ATTCCCTCCTTCAGCAAGA	GGAGAGAGACCAAGTGACTCAGG
<i>Ef-SOD1a</i> Δ	AAGAACCGTATCGGTGACTG	<u>ATGCATTATATCTCCTCTAAAGTTAAC</u>
<i>Ef-SOD2</i> Δ	TCATCTAAGGTTGAGCTTCCAG	<u>ATGCATATATCTCCTCTAAAGTTAAC</u>